3604

Microscopic Method for Determining Shrinkage Temperatures of Collagen and Leather*

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INTRODUCTION

The shrinkage temperature (Ts) is a useful index of the resistance of collagen to swelling in water or water solutions. In general the shrinkage temperature is raised by tanning and lowered by peptizing electrolytes and by protein degradation.

Nageotte and Guyon [7] described a method whereby the shrinkage of reticulin and collagen may be observed in the microscope. Salcedo and Highberger [9], using a heating stage similar to the one described in this paper, noted the shrinkage of formaldehyde tanned collagen fibers. We first used the microscopic method in attempting to relate the ordinary shrinkage properties of collagen to the structure of collagen fibrils, which are so small as to be observable only with the high resolving power of an electron microscope [8]. Lateral swelling of the fibrils, longitudinal shrinkage, and loss of the characteristic striations started at temperatures several degrees below the shrinkage temperature as measured conventionally on a relatively massive specimen [2, 3, 4, 6, 10]. On subdividing the specimen to the extent that magnification of 50 to 100 times was necessary to see the particles (fibers and small fiber bundles), the shrinkage temperature was lowered, approaching the temperature marking the onset of fibril shrinkage as observed with the electron microscope. In linear dimensions, the microscopic particles are about one-thousandth as large as the usual specimens on which shrinkage temperature are measured. As a result, the microscopic shrinkage temperature is unaffected by several factors that may make the conventional shrinkage temperature

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partly characteristic of the specimen rather than of the hide or leather substance. Among the more obvious of these factors are: non-uniform temperature of the specimen owing to its thickness; incomplete or uneven penetration of water during the Ts measurement, caused by the thickness of the specimen and the structure of its fabric; and tension placed on many of the fibers as the fabric swells in the heated water, which increase the temperature at which those fibers shrink.

This paper describes the microscopic method, illustrates its usefulness and its limitations, and presents data comparing Ts values obtained by the microscopical method and the Provisional method of the American Leather Chemists Association [1].

Apparatus and Method

The sample—collagen or leather—comminuted by teasing, grinding or cutting, is suspended in water and passed through a 60 mesh sieve. The material that passes through the sieve is washed or concentrated by centrifuging, if desired, and placed in ordinary capillary melting point tubes. These are sealed in a flame if the Ts is higher than 95°C. In our experience, capillary tubes containing water and thus sealed could be heated to 150°C. or higher without failure. Figure 1 shows the assembled apparatus. The electrically heated microscope stage consists of a duralumin housing mounted on a transite base. The housing is provided with a thermometer, and is drilled to accommodate the melting point tube and to allow light to pass through the specimen. The heating element consists of about 6 feet of No. 26 Nichrome wire wound on an 1/8 inch mandrel. The coil is insulated with sheet mica. The heater is operated at a maximum of 20 volts. The rate of heating is controlled by a variable transformer. Observations are made at an optimum magnification of about 75 diameters.

For measurements at temperatures less than about 85°C., the collagen or leather suspension may be contained between microscope cover glasses placed in a shallow recess in the upper surface of the duralumin housing. Evaporation is retarded by a ring of mineral oil at the periphery of the cover glasses. Use of cover glasses eliminates the lens effect produced by the cylindrical melting point tubes. The apparatus is calibrated separately for the two methods of mounting samples; the transition temperature of sodium sulfate decahydrate and the melting temperature of stearic and benzoic acids are used as thermometric fixed points.

The temperature is noted when the bulk of the suspended material begins to shrink, when marked shrinkage occurs and when the material is completely shrunk. The first and last temperatures constitute the Ts range. The temperature at which marked shrinkage occurs or when particle aggregates shrink is defined as the Ts.

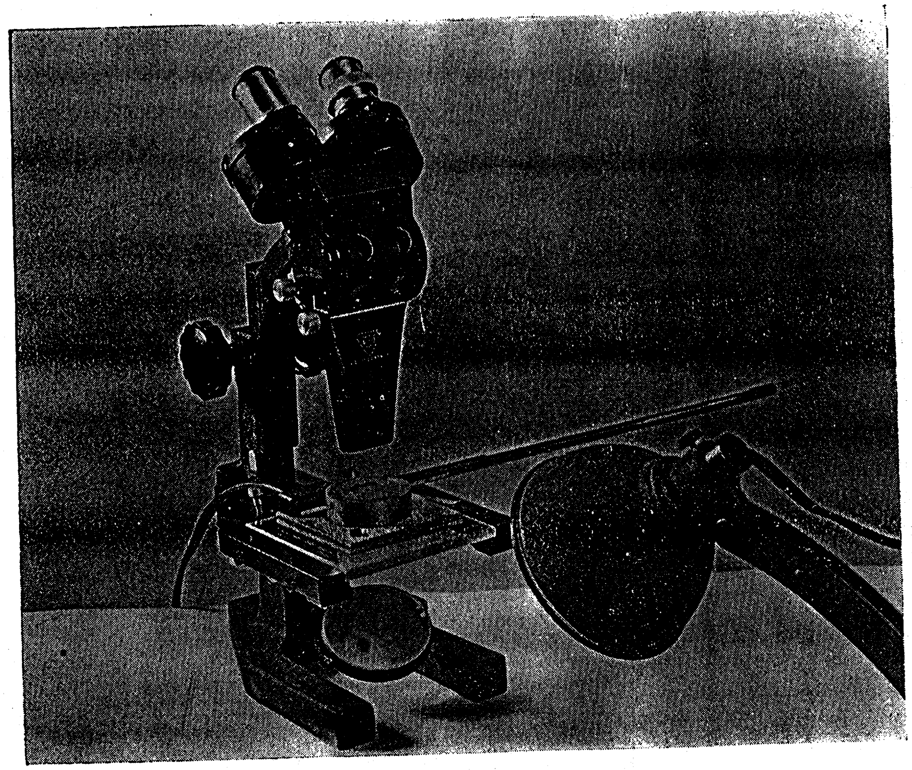

FIGURE 1.—Microshrinkmeter Mounted on Microscope Stage.

Figure 2 is a diagrammatic sketch of the shrinkage reaction of cowhide collagen. At 23.0°C., the start of the determination, collagen fibers represented by the solid diagonal lines are suspended in a cloudy liquor. At 63.0°C. the suspension medium is considerably clarified, and the collagen fibers start to pull away from the sides of the melting point tube. At 65.0°C. the collagen fibers are shrinking quite rapidly; at 65.5°C. the main mass of collagen starts to break up into smaller masses, and at 67.0°C. the small masses are completely consolidated. Marked shrinkage is easily recognized, and inexperienced observers have no difficulty in making the measurement.

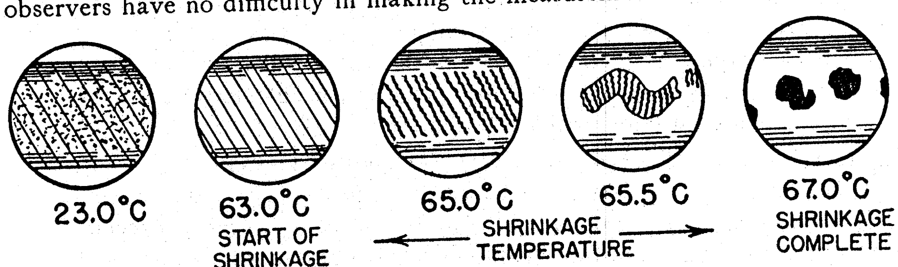

FIGURE 2.—Diagrammatic Sketch of the Shrinking Reaction of Cowhide Collagen.

Observations

Heating Rate and Initial Temperature

Increasing the heating rate raised the Ts measured by the microscopic and by the American Leather Chemists Association methods to about the same extent. This is illustrated by data for a hydrated sharkskin and a tara tanned sheepskin in Table I. Thus, a rate change of from 0.5 to 4°C. per minute increased Ts by 3-4°C. Time may be saved by starting the Ts measurement at an elevated temperature. Tests were made with fresh cowhide, tara tanned sheepskin, chrome tanned calfskin, and sharkskin. By both methods, the Ts was not affected by initial temperature, provided that the initial temperature was 10 degrees or more below the Ts as measured in a control determination started at room temperature as stipulated in the American Leather Chemists Association method. Further saving of time in the microscopic method may be realized by rapidly cooling the duralumin chamber between measurements. This is done conveniently by placing a small lead block, previously cooled in either tap water or ice water, on the chamber.

TABLE I

Effect of Heating Rate on Shrinkage Temperature of Skin and Leather.

Material	Heating rate	Ts	
		Microscopic Method	A.L.C.A. Method
	°C. per Minute	°C.	°C.
Sharkskin	0.5	52.0	54.5
"	4.0	55.0	57.0
Sheepskin, Tara Tanned	0.5	——	68 0
" " "	2.5	64.5	——
" " "	4.0	67.5	72.1
" " "	7.0	69.0	74.5

Particle Size, Concentration

A suspension of cowhide collagen was sieved, and the shrinkage temperature measured on three fractions: (1) material passing through a 60 mesh screen and retained on a 100 mesh screen; (2) passing a 100 mesh screen and retained on a 270 mesh screen; and (3) passing a 270 mesh screen. Ts for the three fractions was equal within 0.5°C. This suggests that a sample of collagen or leather, so ground as to pass a 60 mesh sieve, is suitable for the microscopic Ts measurment without further fractionation.

Changing the concentration of suspended collagen or leather over a 100 fold range did not change the Ts. A concentration effect would be due principally to extractables, and since the whole sample concentration rarely exceeds 0.1 per cent, a change in Ts by the much smaller concentration of dissolved material would not be expected.

Reproducibility of Measurements

In a series of measurements on specimens of an apparently homogeneous sample, there is generally better concordance of Ts values by the microscopic method than by the American Leather Chemists Association method. Also, the microscopic Ts values usually are slightly lower. These findings are possibly relatable to rather subtle physical differences within the microscopic specimens, for example, differences in fabric construction or extent of hydration prior to test. Such inhomogeneity is automatically much reduced in the microscopic specimens. Comparative data for three hide samples and two thin leathers are presented in Table II. It may be noted that fully hydrated test specimens of cowhide cut with the long dimension perpendicular to the backbone line had a Ts spread of only 0.5°C. by the American Leather Chemists Association method. The same material cut with the long dimension parallel to the backbone had a 2°C. Ts spread. The spread for a hydrated sharkskin was again only 0.5°C. Tara tanned sheepskin strips, air-dry at the start of the American Leather Chemists Association test, had a Ts spread of 1°C. So small a spread for this leather may be a consequence of its loose weave which permits ready penetration of water.

TABLE II

Shrinkage Temperature of Skins and Leathers as Determined by the A.L.C.A. and the Microscopic Methods.

			Ts		
			A.L.C.A. Method		
Sample	Weave Pattern	Hydration Prior to Test	Normal Tension	Five Times Normal Tension	Microscopic Method
			°C.	°C.	°C.
Sharkskin	Simple, compact	Complete	57.0–57.5	58.5	55.0–56.0
Cowhide, fresh	Complex, compact; specimens cut perpendicular to backbone line	Complete	68.0–68.5	—	64.0–65.0
	Specimens cut parallel to backbone line	Complete	68.0–70.0	—	64.0–65.0
Cowhide, acetone-extracted	Complex, compact	Rehydrated	68.5–72.0	—	67.0–68.0
Sheepskin, tara tanned	Complex, loose*	Dry	72.0–73.0	74.0	67.0–68.0
Calfskin chrome tanned	Complex, compact*	Dry	77.5–86.5	—	81.0–83.0

*Specimens for the A.L.C.A. test were cut with the long dimension parallel, perpendicular and at 45° to the backbone line.

A section of acetone-extracted cowhide that had been rehydrated by soaking in water until soft and pliable gave a considerably greater Ts spread, from 68.5 to 72.0°C. Some strips tested showed a central streak of incompletely hydrated hide substance. These strips had the higher Ts. Chrome tanned calfskin, which has a compact and complex weave pattern, had a Ts spread of 77.5 to 86.5°C. when the test was begun with air-dry specimens. This large variation cannot be attributed entirely to structural pattern, because there was no grouping of Ts values for strips cut with the long dimension in any particular direction. Rather local compositional differences are postulated. The abnormal spread in the microscopic Ts values is consistent with chemical non-uniformity in the test specimens.

A rough measure of the effect of tension on Ts was obtained for the sheepskin and sharkskin. A 5 fold increase in the tension applied to the strips tested by the American Leather Chemists Association method raised the Ts by 1°C.

The existence of a Ts range by the microscopic method deserves comment, since the particles observed are seemingly free of any constraints imposed by fabric structure or external tension, and are so small that they should be fully hydrated at all stages of the shrinkage measurement For the readily visible particles, a slight gradual shrinkage usually begins perhaps 2 or 3 degrees below a temperature at which sudden, pronounced shrinkage takes place. The latter temperature is designated the shrinkage temperature. During the succeeding interval of 2 or 3 degrees, there is minor additional shrinkage. The shrinkage reaction as a whole is analogous to the melting reaction of a slightly impure material. A microscopic Ts range in this sense is commonly observed for collagen which has not been chemically modified by proteolysis, tannage or any other means. When unequal chemical alteration has taken place, it is evidenced by a series of temperatures at which sudden, marked shrinkage of the suspended particles occurs.

Application to Leather; Stratification

To obtain data on the effect of chemical non-uniformity, Ts measurements were made on cowhide tanned in a quebracho-chestnut liquor. Tannage was purposely incomplete. The leather was washed, dehydrated with acetone and equilibrated at 23°C. and 50 per cent relative humidity. It was cut into 3.5 by 0.5 inch strips, and alternate strips were split by hand into grain, middle and flesh layers. The split strips were trimmed to 3 inches, and the trimmings were dispersed in water to make the samples for the microscopic Ts test. The results are summarized in Table III.

A fair degree of uniformity of tannage within each stratum is indicated by the narrow spread of Ts values by both methods. There was a considerable dispersion of values for the whole leather. Especially with the microscopic method, this is characteristic of inhomogeneous specimens.

Another good example was provided by a group of alum retanned leathers. The Ts of these leathers was measured by the American Leather Chemists Association method, with 75 per cent glycerin as the immersion medium.

TABLE III

Shrinkage Temperature of a Quebracho-Chestnut Tanned Cowhide.

Sample	Ts A.L.C.A. Method	Ts Microscopic Method
	°C.	°C.
Whole leather	74.5 - 79.0	67 - 83
Grain split	86.5 - 87.0	84.5 - 85*
Middle split	73.0 - 76.0	74 - 75
Flesh split	77.0 - 89.0	78 - 82

*This sample was dispersed by a 30 minute treatment in a Waring Blendor; for the less resistant samples, a 3 to 5 minute treatment was sufficient.

Grain, middle and flesh splits were made, and ground separately in a Wiley mill for Al_2O_3 analysis and for microscopic Ts measurement in water. The data in Table IV show a qualitative relationship between Ts and the alum content of the strata. They show also a fairly narrow spread of Ts values for a particular stratum, and the expected wide spread for a composite sample approximating the parent leather in composition.

TABLE IV

Correlation of Al_2O_3 Content of Alum-Retanned Steerhide with Shrinkage Temperature.

No.	Sample Description	Al_2O_3 Content	Ts A.L.C.A. Method*	Ts Microscopic Method**
		Per Cent	°C.	°C.
1	Whole leather	—	98	— - —
	Grain split	2.9	—	95 - 100
	Middle split	0.7	—	90 - 92
	Flesh split	2.9	—	103 - 106
	Composite***	(2.2)	—	93 - 103
2	Whole leather	—	126	— - —
	Grain split	4.6	—	116 - 117
	Middle split	2.5	—	112 - 114
	Flesh split	3.9	—	119 - 120
	Composite***	(3.7)	—	108 - 118
3	Whole leather	—	123	— - —
	Grain split	4.9	—	118 - 119
	Middle split	2.8	—	113 - 114
	Flesh split	4.8	—	117 - 118

*In 75 per cent glycerin.
**In water.
***Equal parts of grain, middle and flesh splits.

The data illustrate the significant advantage of the microscopic method in making easy the measurement of shrinkage temperatures above 100°C. For microscopic specimens shrunk in water alone, temperatures above 100°C.

require pressure apparatus which is inevitably bulky and clumsy to operate. In the American Leather Chemists Association method, the vapor pressure of the immersion medium is reduced to less than one atmosphere of 100°C. by adding glycerin to the water. The greater viscosity of the glycerin-water mixture changes the rate of penetration of the liquid into the specimen, and the swelling power of water for collagen and leather is sometimes changed unpredictably by the addition of glycerin.

Further Application of the Microscopic Method

The microscopic method commends itself because of the rapid evaluation of curing, tanning, or other agents whose effects on collagen or leather may be estimated from the shrinkage temperature. The fine subdivision of the specimen material insures ready accessibility to the reagents and thus a minimum reaction time. Since measurement is made with a microscope, only small quantities of the reacting substances are needed.

As an example, there may be cited a test of the suspected deteriorative effect of silicofluorides on collagen. It has been reported that leather made from hides cured with a sodium chloride—sodium silicofluoride mixture has a lower tensile strength than that from hides cured with sodium chloride alone [5]. Aliquots of a purified cowhide collagen dispersion were treated with 0.3 per cent sodium silicofluoride, (1) alone, (2) with 10 per cent sodium chloride and (3) after addition of sodium hydroxide to pH 5.5. Table V shows that the silicofluoride solution at its natural pH of 3.3 lowered the Ts by 20°C., and swelled and disintegrated the collagen fibrils. At the same pH but in 10 per cent sodium chloride, the Ts was normal, and the fibril appearance was the same as that of the control collagen dispersed in water alone. At pH 5.5, the silicofluoride had a mild tanning action on the collagen, as evidenced by the rise in Ts from 66° to 73°C. From these brief experiments, it appears that silicofluoride added as a preservative to brine does not have a

TABLE V

Effect of Sodium Silicofluoride on Dispersions of Cowhide Collagen.

Treatment	pH	Ts Microscopic Method	Appearance in Electron Microscope
Per Cent		°C.	
None (control)	5.5	66 – 67	Normal, striated fibrils
Na_2SiF_6, 0.3	3.3	46 – 47	Fibrils greatly swollen, in part frayed into non-striated filaments
Na_2SiF_6, 0.3 plus NaCl, 10	3.3	66.5 – 67	Normal
Na_2SiF_6, 0.3	5.5	73 – 73.5	Normal

harmful effect on the fibrils of collagen. The low pH of the silicofluoride solution is attributed to the acid silicofluoride, $NaHSiF_6$. The action at pH 3.3 is explainable as a hydrogen ion effect alone, and this was completely offset by the anti-swelling properties of 10 per cent sodium chloride.

In another experiment, suspensions of cowhide collagen were treated with canaigre tannin, sulfited quebracho tannin and chrome alum. The shrinkage temperature was measured in the tanning liquor after about 5 minutes and again after about 2 hours. Differences were so small as to indicate that tannage was complete in 5 minutes. Portions of the canaigre and chrome tanned suspensions were centrifuged, and the precipitate was washed 4 times with distilled water to remove excess tanning agent. A final determination of Ts in distilled water was then made. The data are summarized in Table VI. The high shrinkage temperature, 98°C., induced by the unusually pure canaigre tannin used, is noteworthy. Since it has been demonstrated that complete tannage requires only a few minutes, the most time-consuming operation is the washing. In the above experiment, the total weight of collagen used was only 0.1 gm. If a larger quantity were used, it would be easily possible to separate the tanned collagen from the tanning liquor by a fine sieve, and to wash it on the sieve. Thereby much time would be saved, and the entire experiment could be completed in little more than an hour.

TABLE VI

Microscopic Shrinkage Temperature of Cowhide Collagen Dispersions Treated with Vegetable and Chrome Tannages.

Treatment	Ts Microscopic Method
	°C.
None (control)	65
Canaigre*, Ts measured in tanning liquor	102
Canaigre*, washed	98
Sulfited quebracho**	90
Chrome alum***, pH 2.5, Ts measured in tannin liquor	40
Chrome alum***, pH 4.5, washed	90

*Canaigre tannin: purity 94.7 per cent; concentration 0.17 g./liter.
**Sulfited quebracho tannin: purity 82.5 per cent; concentration 0.17 g./liter.
***Chrome alum: concentration 15 g./liter.

Standard hide powder (Lot 23) was tested to determine its suitability as a source of finely divided collagen for micro-shrinkage temperature experiments. When suspended in distilled water, the hide powder showed a spread of shrinkage temperature from 45 to 61°C. For a satisfactory material, the spread should be only a degree or two. A suitable collagen powder can be prepared without difficulty from cubes of fresh cowhide corium by washing in sodium chloride solution and then in water, dehydrating in acetone and finally grinding in a Wiley mill. This collagen powder, when suspended in water, has a shrinkage temperature of about 65°C.

Summary

The important factors in the application of a microscopic method to the measurement of shrinkage temperature have been tested. Results are compared with shrinkage temperatures measured by the conventional procedure of the American Leather Chemists Association. The microscopic method has the following advantages: equilibrium with the shrinking medium is assured; owing to the fine subdivision of the samples, rapid tests of tanning materials and other agents used in leather making may be made; effects of structure of skin or leather fabric are eliminated; shrinkage temperatures in water alone may be made conveniently at temperatures up to 140°C. or higher. When dispersions of thick leather specimens are tested, shrinkage over a range of temperatures is generally noted, indicating inhomogeneity such as unequal tannage in the original specimen.

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Discussion

R. M. Koppenhoefer: I think this is a further refinement in our approach to techniques which must be used in the tannery to check the progress of tanning. I think, in its present stage, it would be too involved for routine control purposes; but it is going to be a more valuable asset to our research investigations of leather. Most tannery control work will undoubtedly continue to employ the more rapid and admittedly less exacting techniques. Nevertheless, the contribution is a real one and I think it is a significant advance.

I would like to ask one or two questions of the author. He mentions the use of a sealed tube for running the shrinkage temperatures at temperatures above 100° C. Does he involve any pressure characteristics in this manner to possibly explain the wide differences he got at temperatures over 100? And secondly, he also mentions the use of Tween-20 or a wetting agent such as one of the Tweens. That brings in the same objection as we have to the use of glycerol, namely, glycerol reduces the penetration due to viscosity characteristics; and there are two characteristics involved in penetration, viscosity and wetting ability. Therefore, the use of Tween would affect the second one of the two major factors relating to penetration.

R. BORASKY: The sealed melting point tubes, give us an effective pressure system. The specimens are heated under pressure and the higher shrink temperature in the pressure meter is, I believe, primarily due to size of particles.

As far as Tween-20 is concerned, the concentration of Tween-20 when added, in the amount of one or two drops to approximately a liter of water, is nothing like 75 per cent glycerin. I do not believe the viscosity has been changed too much. The surface tension may be changed quite a bit but here, we illustrate that by emulsifying the fat or increasing the penetration of the water, we get a much sharper reaction.

L. SELIGSBERGER: Is there a certain rule about the ratio between the collagen and the water enclosed in the capillary? Have different results been obtained by changing the ratio?

BORASKY: I do not believe the concentration of the collagen in suspension affects the shrinkage temperature. The method works equally well with a concentrated suspension or with just a few test fibers in the same amount of water. The solid-volume relationships, are of a similar order to the relationships that you get with the Theis meter. You have about three grams of leather per liter of water, and here you have an infinitesimal amount of collagen but also a very small volume of water. I do not believe that affects the shrink temperature any at all.

F. O. SCHMITT: I am sure what Dr. Koppenhoefer said is true, that this is a valuable research method. We have used it ourselves, and it does eliminate certain factors. But the tanner is interested in the properties of the leather. And what you do here is to remove a very small segment from the skin and determine more accurately, perhaps, and under better conditions the apparent shrinkage temperature. Other methods, which use the whole skin, give a better index to the tanner, if the method could be made more suitable scientifically. I have always thought that perhaps the diachrometric method might solve that problem if one could arrange to get equilibration with temperatures suitably rapid. We certainly have found differences in the properties of collagen, whether from tanned or untanned skin, when you get small enough pieces. It is different than before fragmentation.

M. MAESER: As far as running a shrink temperature in the tannery is concerned, I see one rather big difficulty. The shrink temperature of the grain of leather and the shrink temperature of the layer immediately under the grain, and the shrink temperature of the flesh leather or that of the corium are quite different than for the whole skin. All you have to do is to split a skin and run a shrink temperature on the various layers and you may find variations as great as fifteen degrees. Here a few isolated particles of the skin are used. It will be difficult to tell what particular particle has been picked out to represent the whole skin.

BORASKY: May I state this method will not give you an overall picture of shrinkage but I believe we can overcome the objection of Mr. Maeser in that the method of preparation, I think, gives a very valid sample of the entire thickness of the skin and would get a representative shrink temperature. I will admit that the shrinkage properties of intact skin are quite different than they would be on individual fibers or fibrils.

KOPPENHOEFER: This method was intended mainly, was it not, as a research tool refinement? I do not think you visualized carrying it on into plant control purposes.

BORASKY: No.